A NOTE ON CHANGES IN SULFHYDRYL CONTENT DURING MIXING OF DOUGHS CONTAINING NONFAT DRY MILK[1]

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The sulfhydryl groups of flour proteins influence dough mixing and baking characteristics of flours (1–5). They are oxidized during dough mixing, and the rate of decrease in sulfhydryl content differs among flours (6).

Milk proteins retain some sulfhydryl groups, even after the heat-treatment required to prepare nonfat dry milk (NFDM), suitable for bread-baking purposes, and these sulfhydryl groups may differ in reactivity from those in flour proteins. If the possibility of sulfhydryl-disulfide interchange reaction between flour and milk proteins in doughs is conceded, additional sulfhydryl groups could be formed in the milk proteins during dough mixing.

To determine whether milk solids are involved in sulfhydryl losses in doughs, the comparisons reported below were made. Effects attributable to NFDM were small but consistent. When moderate amounts of NFDM (3–6% of flour weight) were added to doughs, generally fewer sulfhydryl groups were lost during mixing than in the absence of milk solids. When a large amount (15%) was added, however, the effect was reversed.

Four flours employed commercially in white bread production by continuous mix processes were used. Flour D is considered less tolerant

[1]Manuscript received July 5, 1963. Presented at the 48th annual meeting, April-May 1963, Minneapolis, Minnesota. Contribution from the Western Regional Research Laboratory, Albany, California. This is a laboratory of the Western Utilization Research and Development Division, U.S. Department of Agriculture, Albany 10, California.

to NFDM than the other flours. The NFDM used, a commercial high-heat product, had a moisture content of 3.4% and a pH of 6.5 when reconstituted. A Harland-Ashworth value of 0.7 mg. whey protein N per g. was obtained (7–9). Protein and sulfhydryl values are given in Table I.

TABLE I
PROTEIN AND SULFHYDRYL CONTENTS (DRY BASIS) OF MATERIALS

	PROTEIN	SULFHYDRYL
	%	μeq./g.
Flour A (N × 5.7)	16.8	1.01
Flour B	14.4	0.95
Flour C	14.8	0.95
Flour D	14.1	0.80
Nonfat dry milk (N × 6.38)	38.0	1.39

Doughs were mixed 20 min. in a 50-g. farinograph bowl with normal exposure to air, then frozen on dry ice and freeze-dried as described previously (6). NFDM and sodium chloride were mixed dry with flour in the farinograph before liquids were added. The amount of acetic acid required to reduce dough pH to 5 was determined by preliminary trials. It was added to the water used in doughs. Doughs contained 50 g. flour (14% moisture basis), 1 g. salt, and 1.5, 3.0, or 7.5 g. NFDM (containing 3.4% moisture).

Sulfhydryl determinations (10) were made by amperometric titration with silver nitrate in tris buffer-urea suspensions (11). Each value reported is based on three or four titrations. When three titrations were made, sample sizes were 200, 400, and 600 mg.; when four titrations were made, an 800-mg. sample was included. From the titers, the slope of the line representing ml. titrant per g. sample was calculated by a least-squares method.

Obviously the titrations could not establish the source of reacted sulfhydryl (–SH) groups, whether from flour or milk. The losses of –SH groups therefore have been presented in two ways. First, the total –SH content of each flour-NFDM combination and the residual –SH content of the corresponding doughs were used to calculate the percentage of –SH groups lost. Results are presented in Fig. 1, the points shown being the mean values for all four flours. At pH 5, the percentage loss decreases at least through the 6% NFDM level, but the trend reverses before the 15% level is reached. With the pH unadjusted, a similar but less marked trend appears to be present, although the absence of a value at 6% NFDM makes it less certain.

Comparison of results on a percentage loss basis was not considered entirely satisfactory, because the total sulfhydryl content increases

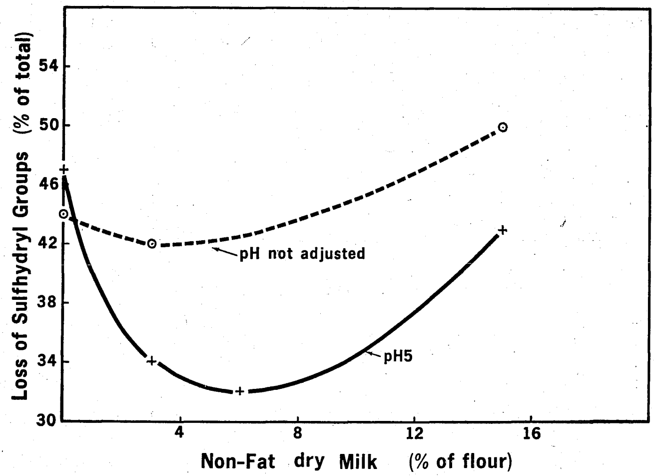

Fig. 1. Effect of added nonfat dry milk on loss of sulfhydryl groups during dough mixing. Values are averages of four baker's bread flours. Doughs containing 2% salt were mixed 20 min. in a farinograph, 30°C., in air; pH 5 obtained by addition of acetic acid. (Averages have been used to illustrate the principal trends. Although the individual flours differ appreciably in percentage losses at each NFDM level as shown in Table II, the trends with all flours are similar.)

with the NFDM level, while at the same time the concentrations of flour components responsible for some –SH loss (e.g., free fatty acids and lipoxidase, 5) are diluted appreciably. For a second type of comparison, therefore, the original –SH contribution of the added NFDM was subtracted from the total found in each dough, and the residual –SH was calculated to a "1-g. dry flour" basis. From these results and the sulfhydryl contents of the original flours, the losses of –SH in μeq. per g. flour were determined and are given in Table II.

TABLE II

EFFECTS OF NONFAT DRY MILK (NFDM) AND OTHER ADDITIONS ON SULFHYDRYL LOSSES IN DOUGHS[a]

Additions to Flour-Water Dough		Sulfhydryl Lost			
		Flour A	Flour B	Flour C	Flour D
		μeq.	μeq.	μeq.	μeq.
None		0.43	0.42	0.34	0.29
2% Salt		0.48	0.48	0.38	0.30
2% Salt + 3% NFDM		0.41	0.42	0.46	0.36
+15% NFDM		0.63	0.63	0.56	0.49
2% Salt and acetic acid to pH 5	0% NFDM	0.47	0.48	0.43	0.35
	+ 3% NFDM	0.38	0.39	0.30	0.26
	+ 6% NFDM	0.34	0.42	0.30	0.26
	+15% NFDM	0.52	0.54	0.50	0.42

[a] Doughs were mixed 20 min. in a farinograph, 30°C., with normal exposure to air. The –SH contents of the dough were determined, calculated to 1-g. dry flour basis, and then subtracted from the –SH contents of the corresponding flour to obtain the values given here.

Both methods of expressing the results show the same trends, because the contribution of NFDM to the –SH content in all mixtures was relatively small, the maximum being about one-fifth. (15% NFDM added to flour D increased –SH content of the mixture by 0.197 μeq. per g. over that of flour D alone.) However, the results in Table II show that the absolute –SH loss per g. of flour at 15% NFDM and pH 5 is larger than with 0% NFDM, although the percentage loss is smaller.

Examination of the results in Table II also shows that with the doughs at pH 5 all flours retained more –SH groups in the presence of 3 and 6% NFDM than in the control. Thus there is no indication that –SH groups in NFDM are oxidized at pH 5 during dough mixing; instead, it seems reasonable to conclude that more flour –SH groups are retained. When the much larger additions (15%) of NFDM were made, decreases in –SH content did exceed those in the control doughs. This suggests that –SH groups in both milk and flour proteins were lost. Among the doughs not adjusted in pH, the C and D doughs containing 3% NFDM did not retain more –SH than their controls, but it appears that the losses were unusually small in the controls rather than large in the 3% NFDM doughs. With 15% NFDM, pH unadjusted, losses were the largest observed with all flours.

While the trends observed are quite consistent, the absolute differences are small. Therefore, to help evaluate the significance of the results, an analysis of variance was carried out, and Duncan's multiple range test was applied to the differences between treatment means using the data for –SH content of the flour-NFDM mixtures and doughs, in μeq. per g. flour. The results confirmed the trends shown in Fig. 1. That is, at pH 5 the control and 15% NFDM means differed significantly (5% level) from the 3 and 6% NFDM means. With unadjusted pH, the 15% NFDM mean differed from all others. Some of the individual values (Table II) suggest that interactions of flours and NFDM may occur, but the available experimental results were not sufficient to resolve the point.

With 3 and 6% added NFDM, farinograph curves showed increased stability while retaining a normal shape. With 15% milk solids, however, the curve shape was markedly altered; the band was narrow, and resistance to mixing increased only slowly for several minutes. These changes were apparent at pH 5, but were even more marked with no control of pH.

Several factors may be involved in the effects of NFDM on –SH losses. The presence of salts, particularly of heavy metals, in the NFDM may accelerate losses. The change in physical properties of the doughs containing 15% NFDM may also be reflected in the loss of sulfhydryl groups if it results in increased incorporation of oxygen or in greater exposure of sulfhydryl groups in the flour proteins. On the other hand, the observed oxidation of –SH groups by oxidized fatty acids (5) and the known protection of –SH groups by antioxidants added to doughs suggest that oxidized lipid material in doughs may react with easily oxidized constituents of NFDM, other than –SH groups, and so protect –SH groups of flour proteins. It is also possible that some associations of milk and flour proteins protect –SH groups in both groups of proteins; then at high levels of NFDM the capacity of wheat proteins for such combinations would be exceeded and the milk –SH groups would react readily. The reversal of the effect in comparing low and high levels of NFDM suggests that some of these factors may be dominant at low and others at high NFDM levels.

The system obviously is complex, so that more information is needed to evaluate the influence of each of the factors suggested. However, the pH-buffering and water-absorptive properties of NFDM are well recognized but do not account for all the effects of NFDM in baking. The results given above therefore have been presented at this stage to demonstrate another property of NFDM that may be significant.

Acknowledgment

The authors are indebted to Robert H. Cotton and Stanley T. Titcomb, Continental Baking Company, Rye, New York, for the flours used in this work.

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